PATENT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Applicant:	Grabowski and Du	: Paper No:	
Serial No.	09/775,517	: Group Art Unit:	1651
Filed:	February 2, 2001	: Examiner:	Jon Weber
For:	LIPID HYDROLYSIS THERAPY FOR ATHEROSCLEROSIS AND RELATED DISEASES		

DECLARATION UNDER 37 C.F.R. 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

David Hui Ph.D., declares that:

Considered
27 May 2004

1) I am a Professor of Pathology & Laboratory Medicine at the University of Cincinnati. I received a Ph.D. in Biochemistry from Indiana University in 1979. I have worked at the University of Cincinnati since 1987. My areas of expertise include utilization of cellular, molecular, and genetic approaches to explore the structure and function of lipid transport proteins, as well as their roles in normal physiology and pathophysiologic conditions such as coronary artery disease, diabetes and obesity, hyperlipidmia, and nutrient digestion and transport.

2) I am familiar with the Grabowski and Du lysosomal acid lipase (LAL) therapy patent application, which describes a method to diminish and/or eliminate atherosclerotic plaques, in mammals, through direct and indirect treatment of these plaques, *in situ*, using proteins and/or polypeptides that are capable of lipid removal, primarily through hydrolysis. The lipid hydrolyzing protein or polypeptide targets receptors in and/or on the cell which lead to uptake into the

lysosome. Compositions used for practicing this invention include lipid hydrolyzing proteins or polypeptides, and in particular, lysosomal acid lipase (LAL).

3) A hallmark of atherosclerosis is the deposition of cholesterol and cholesterol-rich macrophage foam cells in the arterial wall. Increased circulating levels of low density lipoproteins (LDL) as well as decreased level of high density lipoproteins (HDL) are major risk factors for premature atherosclerosis. The mechanism by which increased LDL level contributes to atherosclerosis is well established. The prolonged circulation of LDL results in their increased susceptibility to oxidative modification and the oxidized LDL can be internalized into macrophages by a non-regulated scavenger receptor mediated endocytotic pathway. The internalized oxLDL are delivered to the lysosomes where the cholesteryl esters associated with the lipoproteins are hydrolyzed by lysosomal acid lipase (LAL), the unesterified cholesterol liberated from this hydrolysis exits the lysosomes where it can be esterified into cholesteryl esters by acyl-CoA:cholesterol acyltransferase (ACAT). The cholesteryl esters are stored in the cytoplasm as lipid droplets and the accumulation of these droplets result in foam cell formation.

4) I have read and am familiar with the Grabowski and Du patent application. In the Grabowski and Du patent application, LAL is used as the enzyme to promote cholesteryl ester hydrolysis. Note that LAL and HSL have distinct intracellular location and presumed to have distinct functions in intracellular cholesterol homeostasis. Whereas HSL in a cytosolic enzyme, thus capable of participating in hydrolyzing the cytoplasmic cholesteryl ester droplets in macrophage to limit foam cell formation, LAL is a lysosomal enzyme that participates in liberating

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unesterified cholesterol from cholesteryl esters entering the cells through endocytosis.

5) In my scientific opinion, I believe that the exogenous LAL therapy as described by Grabowski and Du, in all likelihood should not work to clear atheroslerotic plaque, and if it did work as stated, it would have limited utility. Furthermore, if this LAL therapy is shown to successfully clear atherosclerotic plaque, such results would be wholly unexpected and surprising given the current state of research in this field.

6) I hereby declare that all statements made herein of my own knowledge are true and that all statements made on the information and belief are believed to be true; and further that these statements are made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or any patent issued therein.

David Hui, Ph.D. 3/26/04

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The Role of LAL

Common pathway to control of cholesterol and triglyceride metabolism

The Metabolic Role of LAL

Hydrolysis of cholesteryl esters and triglycerides delivered to Lysosome by the LDL receptor-mediated endocytotic pathway

Liberated free cholesterol and fatty acids signal many downstream events

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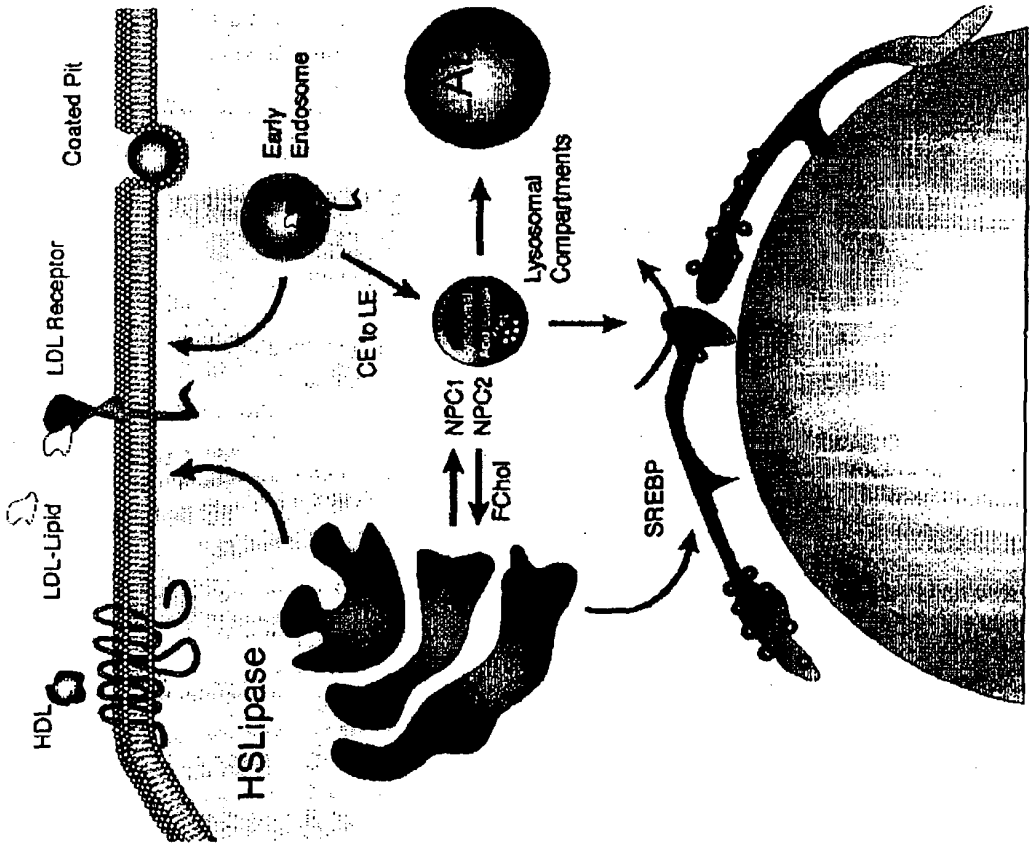
The Metabolic Role of Hormone Sensitive Lipase (HSLipase)

Hydrolysis of cholesteryl esters delivered to the Cytoplasm by HDL mediated transfer

Liberated free cholesterol is re-esterified


Coated Pit
Early Endosome
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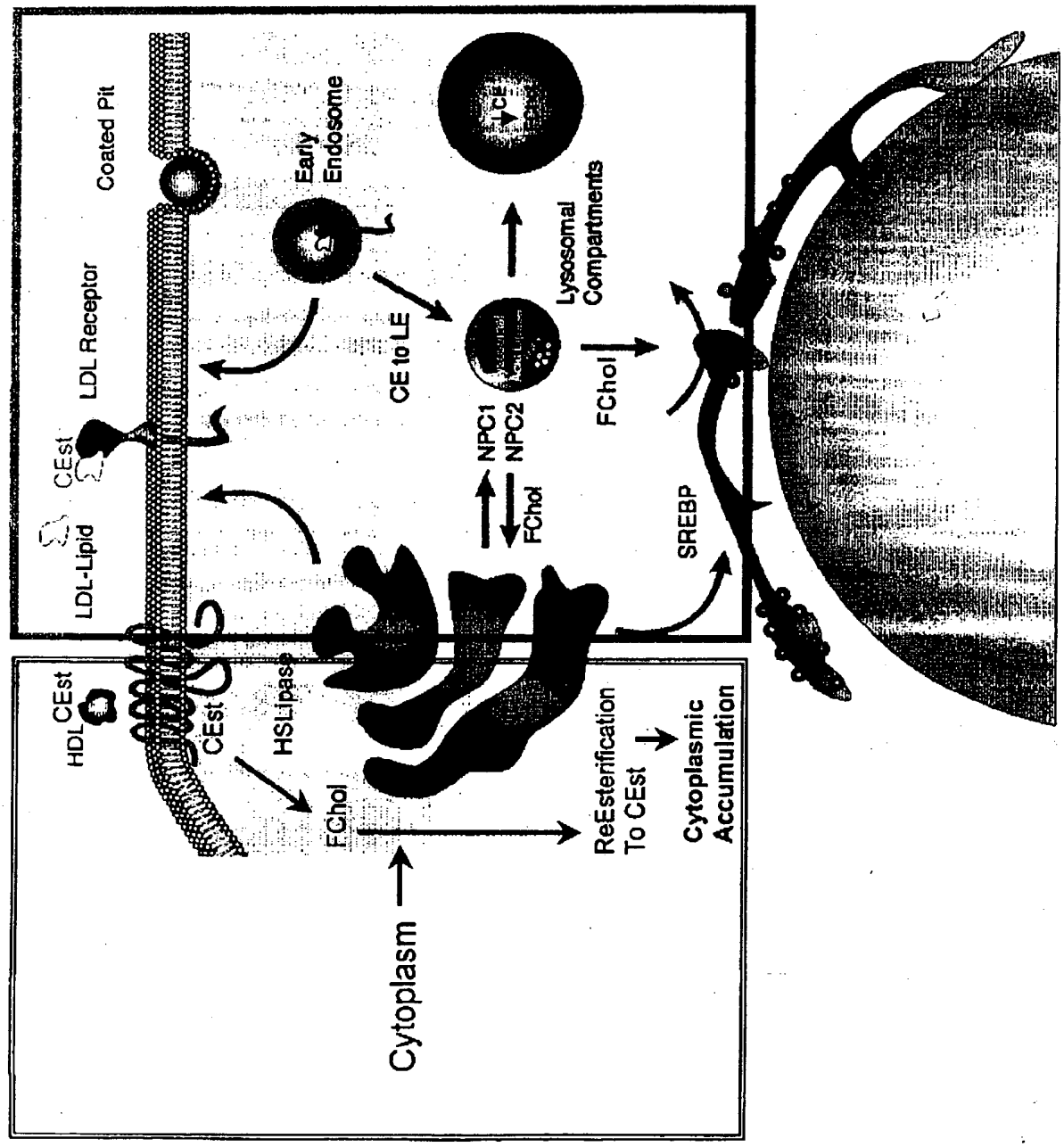
CE to LE
Lysosomal Compartments
LDL-Lipid
NPC1
NPC2
FChol
SREBP
HDL
HSLipase


Coated Pit
Early Endosome
LDL Receptor
CE to LE
Lysosomal Compartments
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CEst
LDL-Lipid
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NPC2
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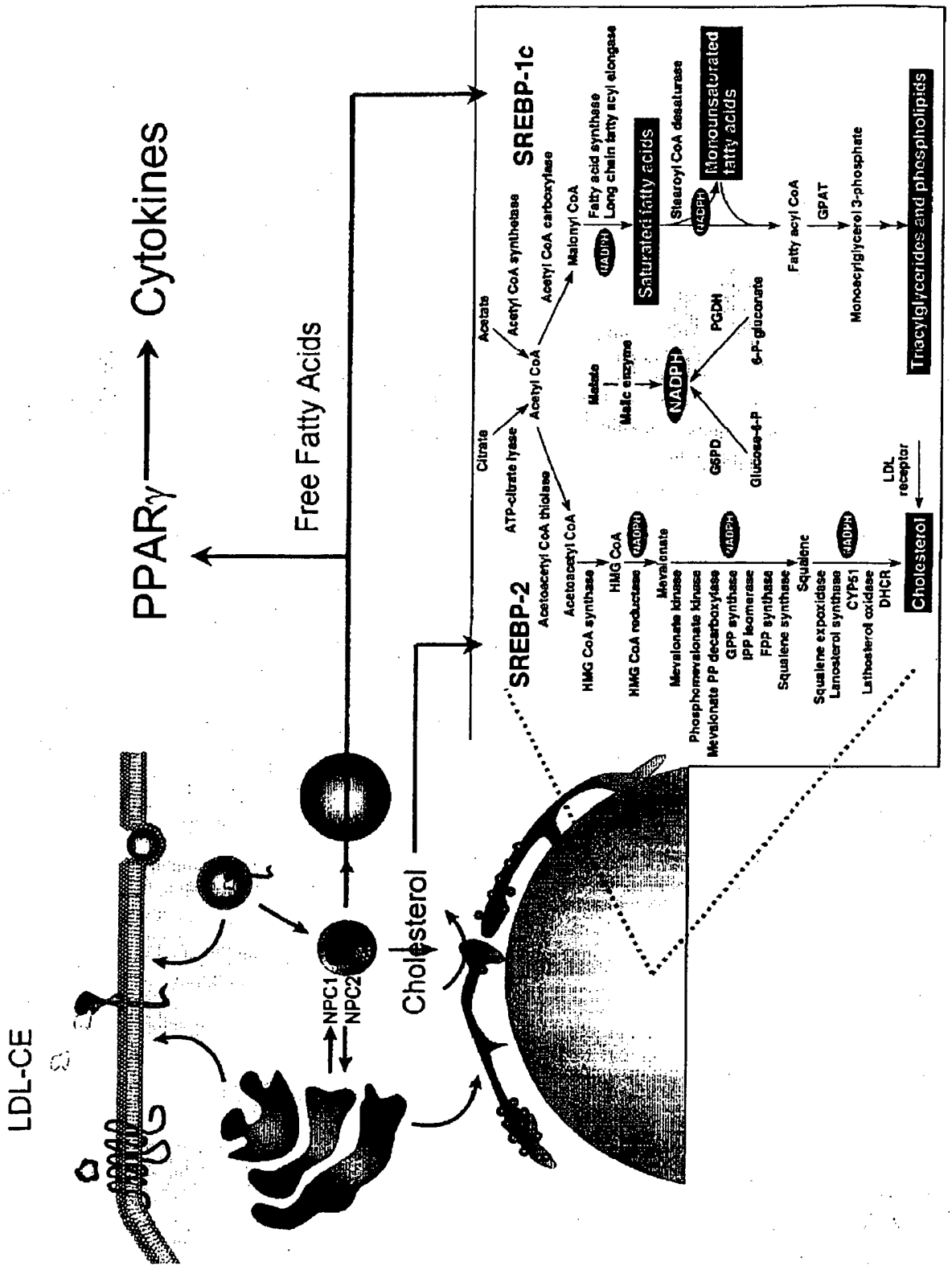
HDL
CEst
CEst
HSLipase
FChol
ReEsterification To CEst
Cytoplasmic Accumulation
Cytoplasm


LDL-CE
NPC1
NPC2
Cholesterol
PPARγ
Cytokines
Free Fatty Acids
SREBP-1c
SREBP-2
Acetate
Acetyl CoA synthetase
Acetyl CoA carboxylase
Malonyl CoA
Fatty acid synthase
Long chain fatty acyl elongase
Saturated fatty acids
Stearoyl CoA desaturase
Monounsaturated fatty acids
Fatty acyl CoA
GPAT
Monoacylglycerol 3-phosphate
Triacylglycerides and phospholipids
Acetyl CoA
Citrate
ATP-citrate lyase
Malate
Malic enzyme
NADPH
PGDH
6-P- gluconate
G6PD
Glucose-6-P
Acetoacetyl CoA thiolase
Acetoacetyl CoA
HMG CoA synthase
HMG CoA
HMG CoA reductase
Mevalonate
Mevalonate kinase
Phosphomevalonate kinase
Mevalonate PP decarboxylase
GPP synthase
IPP isomerase
FPP synthase
Squalene synthase
Squalene
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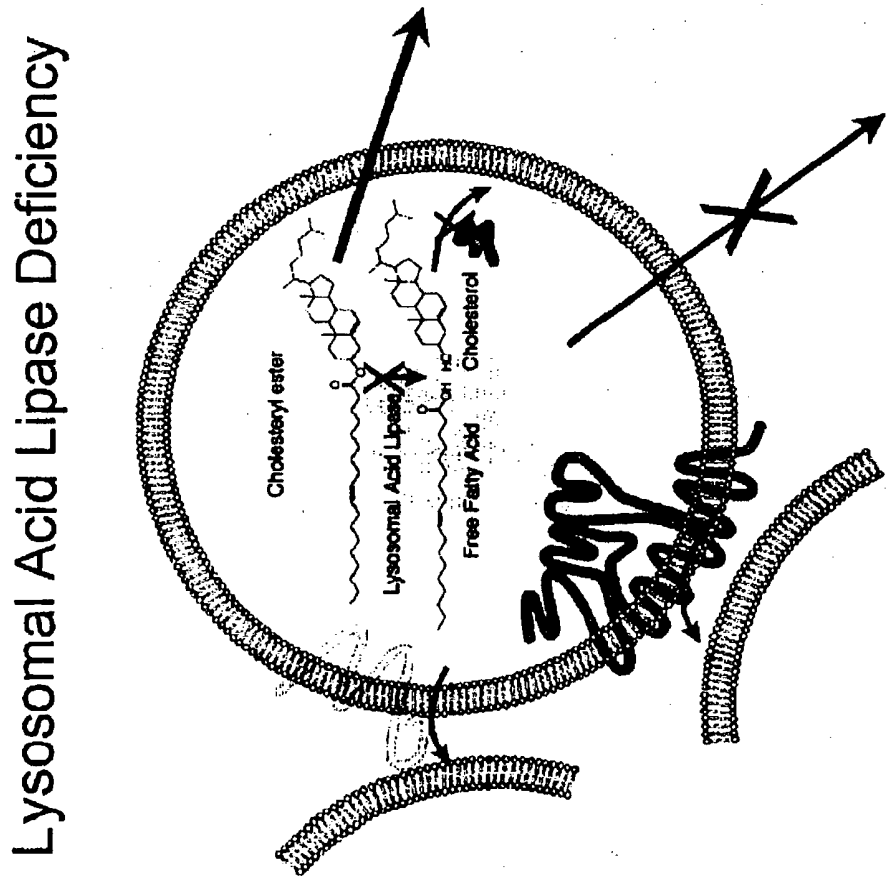
Lanosterol synthase
CYP51
Lathosterol oxidase
DHCR
Cholesterol
LDL receptor


Lysosomal Acid Lipase Deficiency
Cholesteryl ester
Lysosomal Acid Lipase
Free Fatty Acid
Cholesterol

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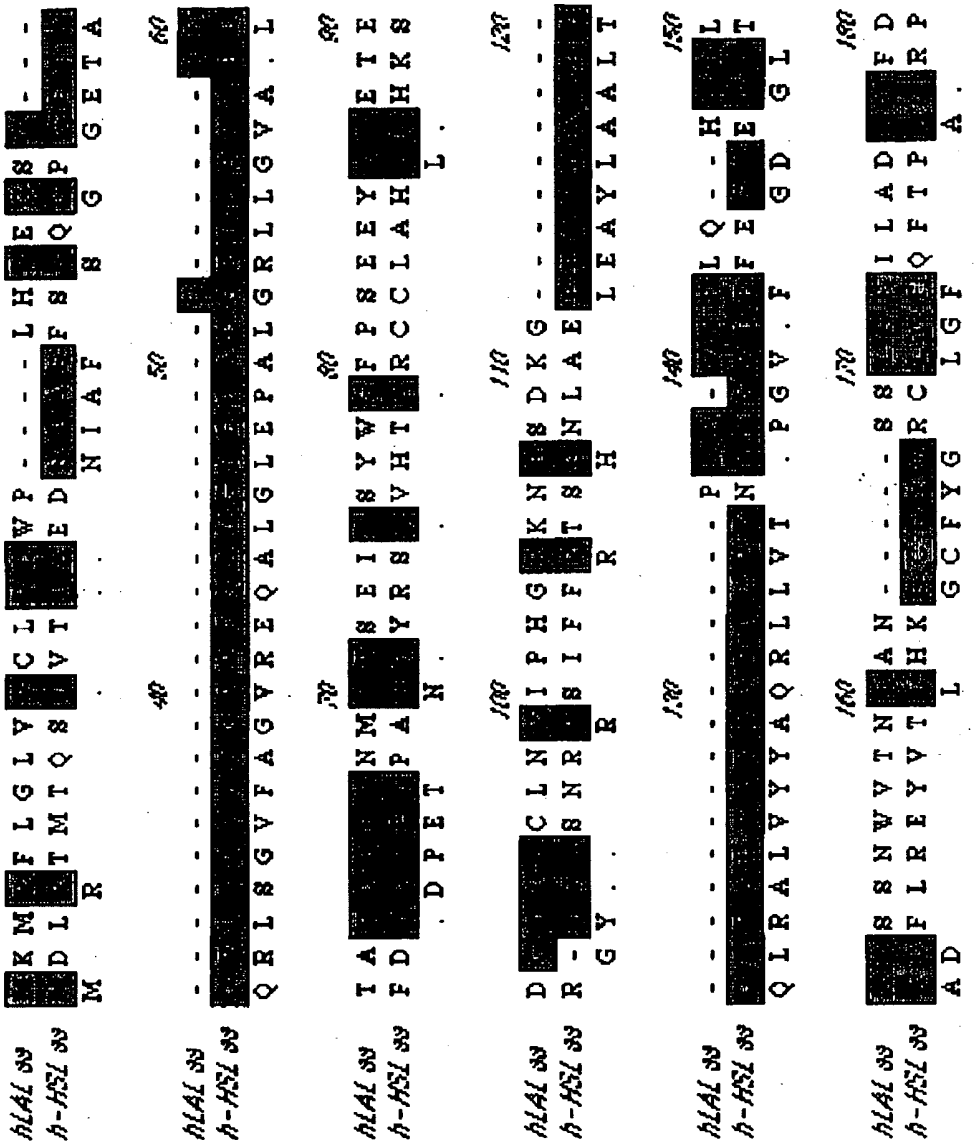
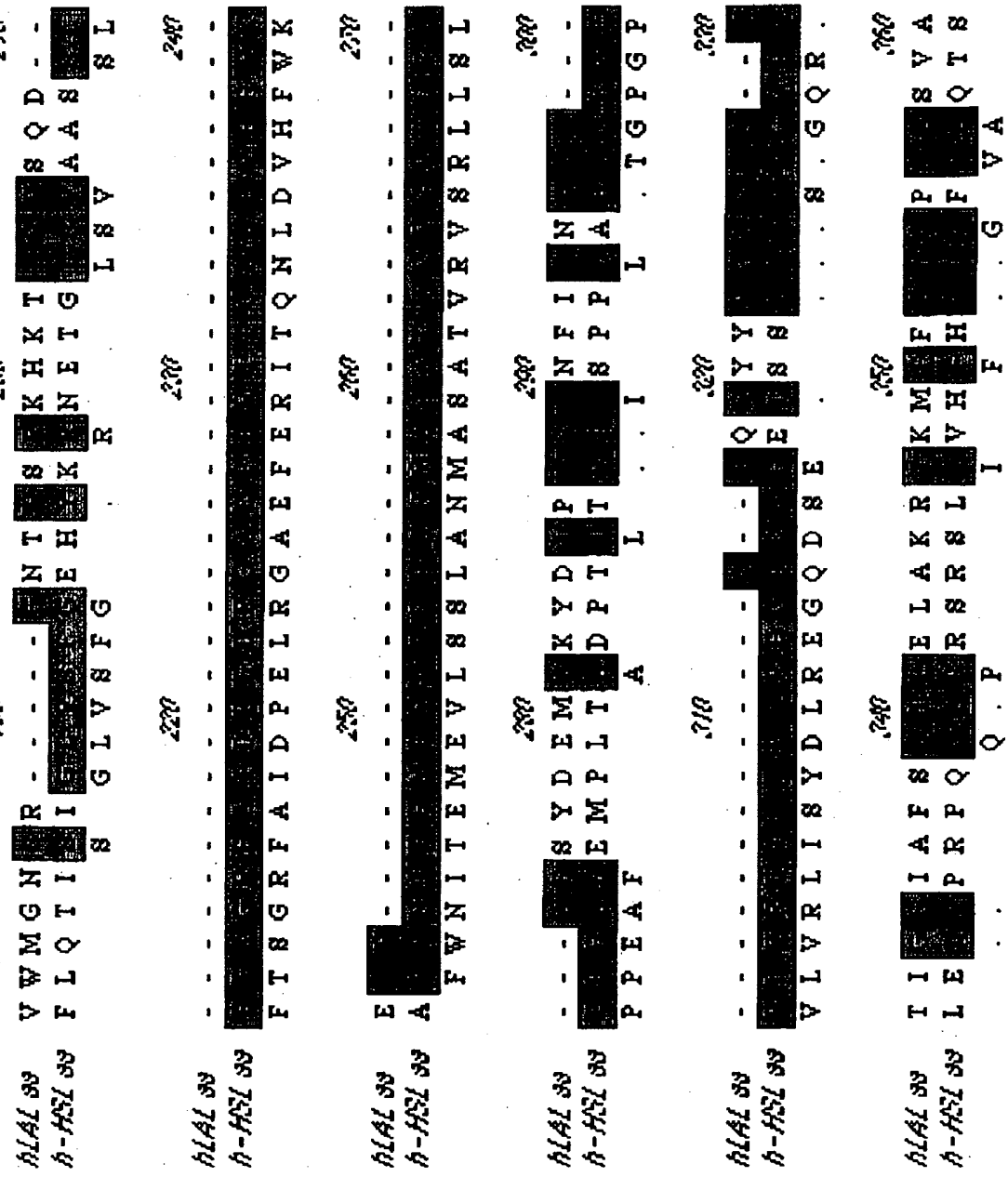
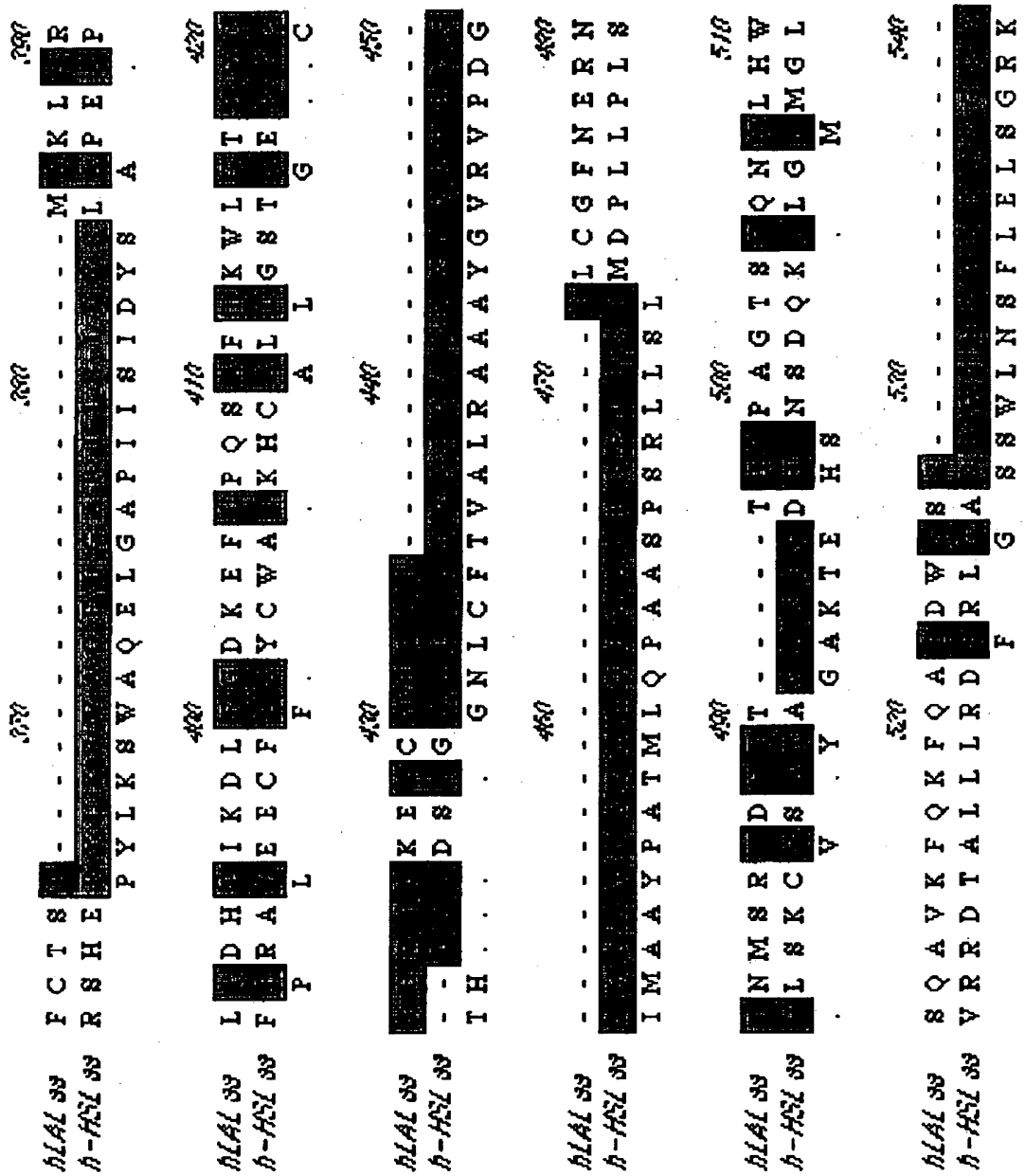
LAL and HSLipase Are Different Enzymes

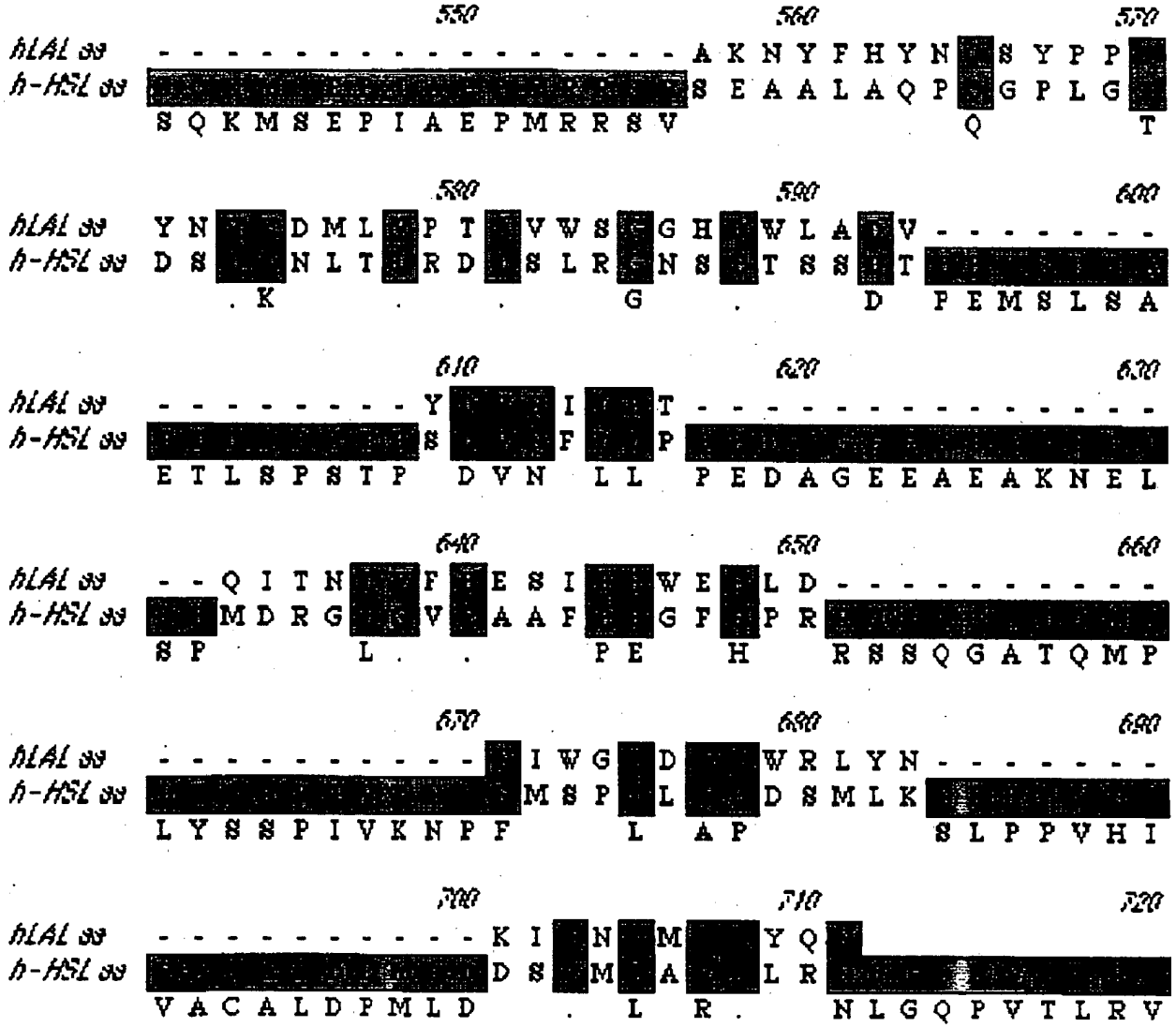
- Different cell compartments
  - LAL in lysosomes
  - HSLipase in cytoplasm
- Different amino acid sequences
- Different genes
- Different post-translational modifications
- Different developmental/tissue expression
- Different functions in cell

Amino Acid Alignment (hLAL and h-HSL)





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hLAL aa
h-HSL aa
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